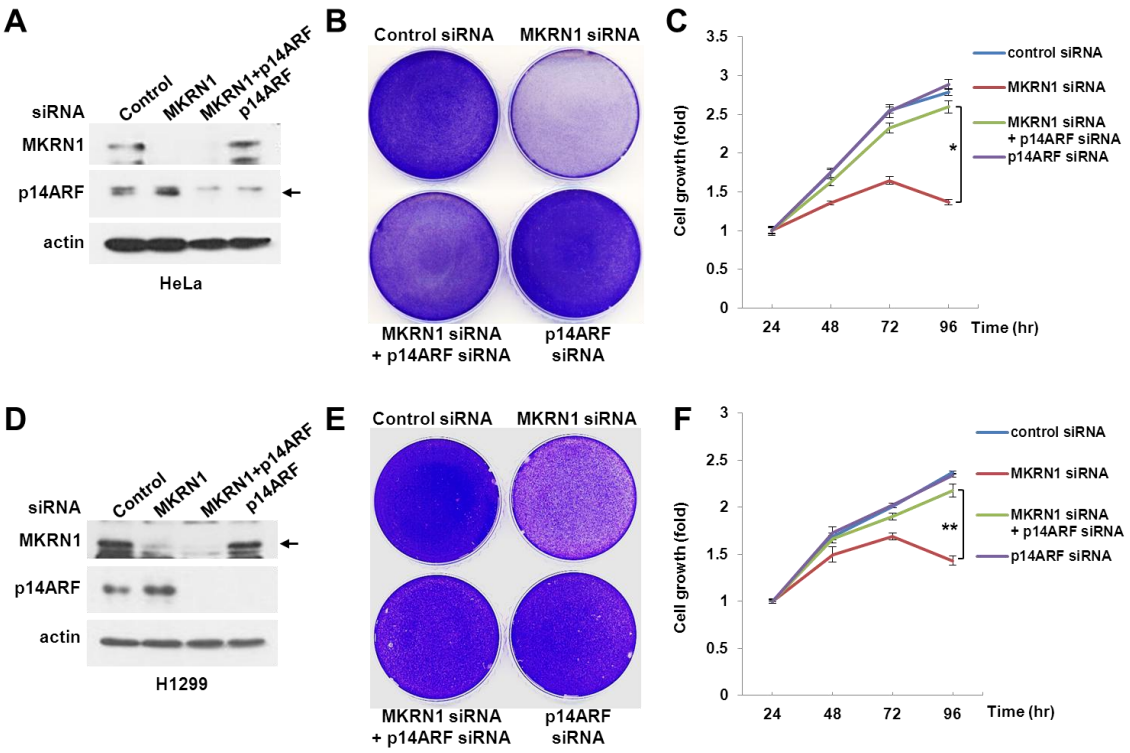
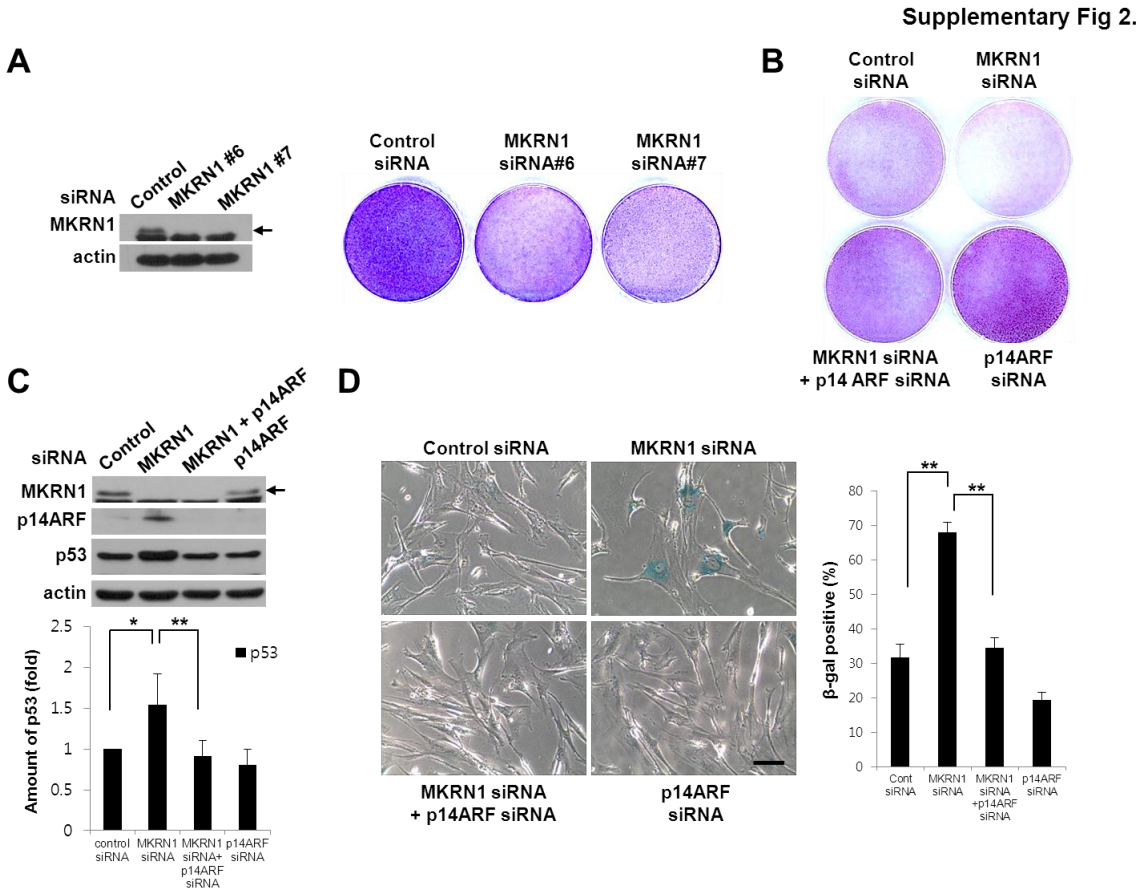
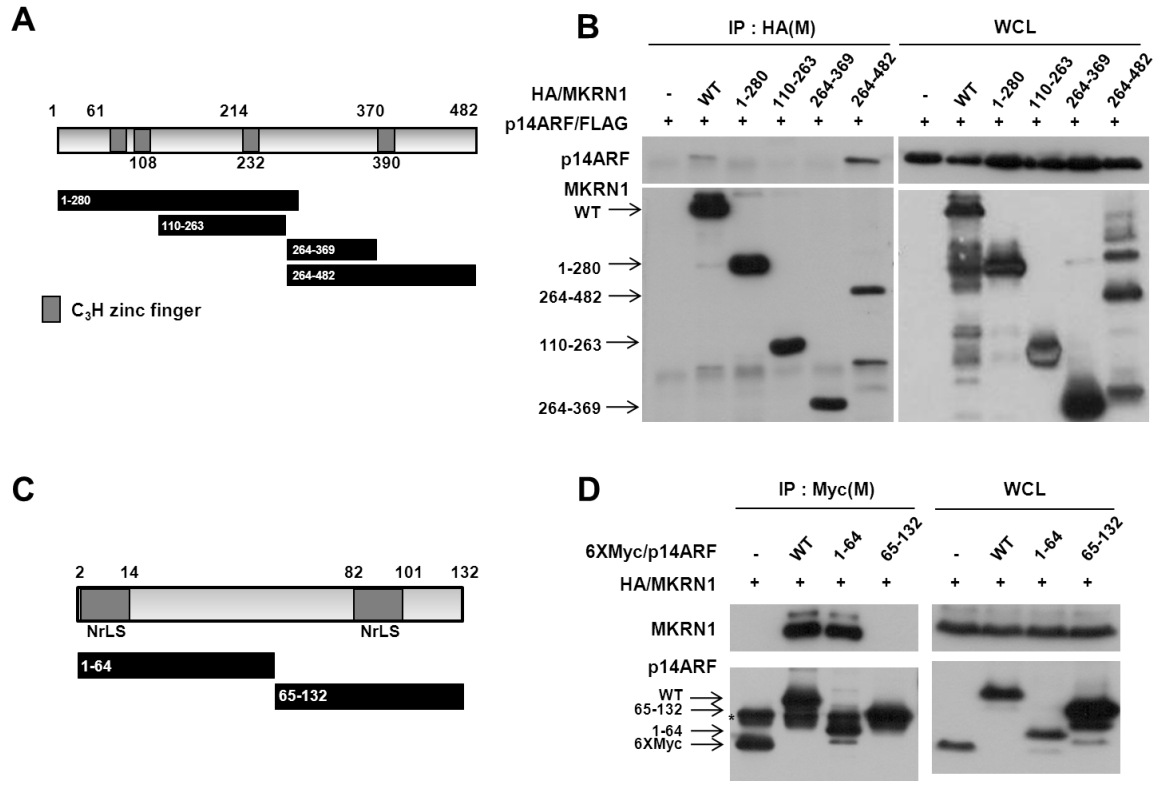


Supplementary Figure 1. MKRN1 inhibits p14ARF-induced and p53-independent cell death. (A) p14ARF protein is stabilized by MKRN1 depletion in HeLa cells. HeLa cells were treated as indicated in Figure 1A and their lysates were analyzed by immunoblotting with antibodies to p14ARF, MKRN1, and actin. (B) p14ARF depletion blocks inhibition of cell proliferation induced by MKRN1 ablation in HeLa cells. HeLa cells were transfected with control or MKRN1 #6 siRNA with or without p14ARF siRNA as indicated. After 96 hr, cells were stained with crystal violet. (C) Ablation of p14ARF rescued the cell growth retardation induced by MKRN1 depletion. HeLa cells were transfected with siRNA as indicated. After 24, 48, 72, and 96 hr, cell viability was assayed using CellTiter-Glo® Reagent. The error bars indicate 95% confidence intervals of three experiments. (D) p14ARF protein is stabilized by MKRN1 depletion in H1299 cells. (E) p14ARF depletion blocks the inhibition of cell proliferation induced by MKRN1 ablation in H1299 cells. (F) Ablation of p14ARF rescued the cell growth retardation induced by MKRN1 depletion in H1299 cells. D-F were carried

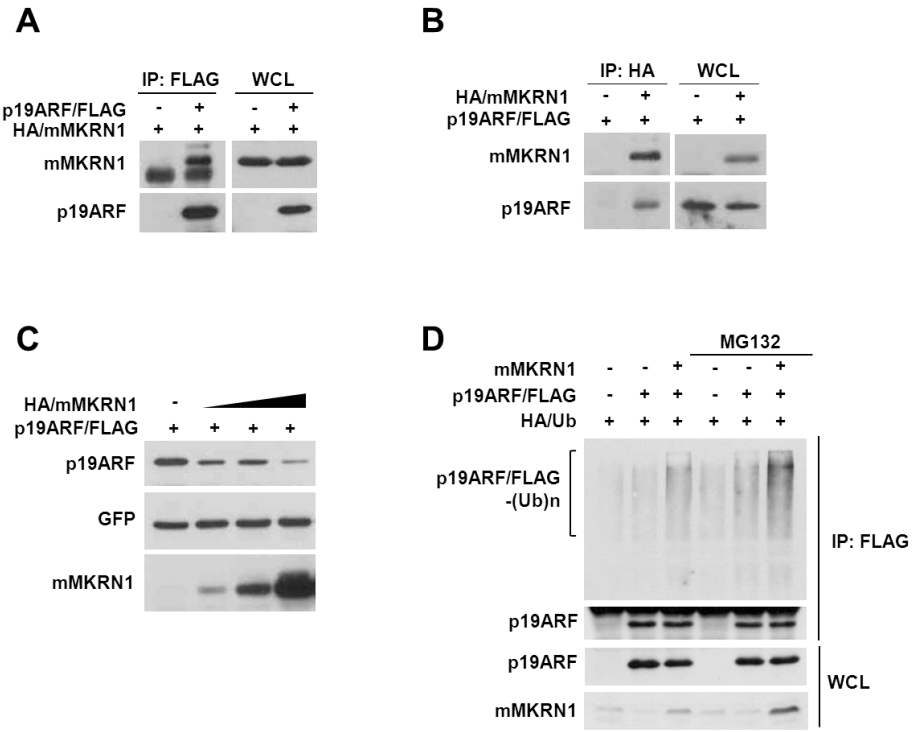
out according to the same methods as A-C. *: p =.008, **: p =.005. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading frame.



Supplementary Figure 2. Knock-down of MKRN1 induces cell cycle arrest and senescence through stabilization of p14ARF in IMR-90 fibroblasts. (A) Knock-down of MKRN1 suppresses growth of IMR-90 cells. (B) p14ARF depletion blocks the inhibition of INR90 cell growth induced by MKRN1 ablation. (C) Knock-down of MKRN1 induces stabilization of p14ARF. (D) Cellular senescence induced by MKRN1 depletion is reversed by p14ARF ablation. All experiments were carried out as described in Figure 1. The arrows indicate MKRN1 proteins. *:p =.039, **: p <.001. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading frame.

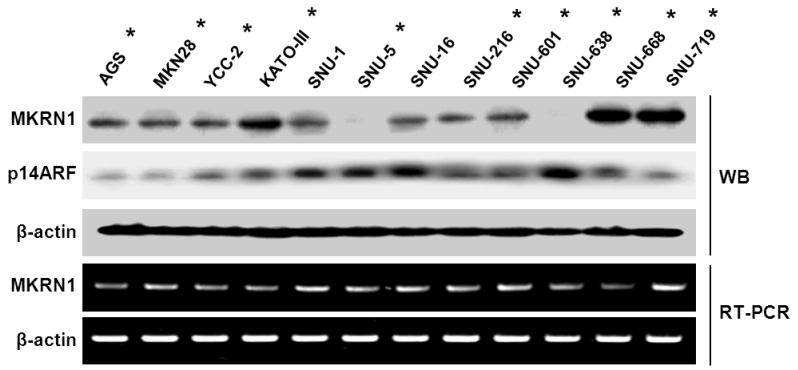


Supplementary Figure 3. The C-terminal of MKRN1 and N-terminal of p14ARF are required for interaction. (A) Diagram of MKRN1 deletion constructs. (B) The C-terminal region of MKRN1 interacts with p14ARF. Plasmids expressing p14ARF/FLAG and HA/MKRN1 or its truncation mutants were transfected into 293T cells. WCL were immunoprecipitated with anti-HA antibodies and detected using anti-FLAG or HA antibodies. (C) Diagram of p14ARF deletion constructs. (D) The N-terminal region of p14ARF is required for its interaction with MKRN1. Plasmids expressing HA/MKRN1 and 6XMYC/p14ARF WT or its deletion mutants were transfected into 293 cells. WCL were immunoprecipitated with anti-c-Myc antibodies and detected with anti-HA or c-Myc antibodies. The asterisk indicates light-chain antibodies. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading frame.

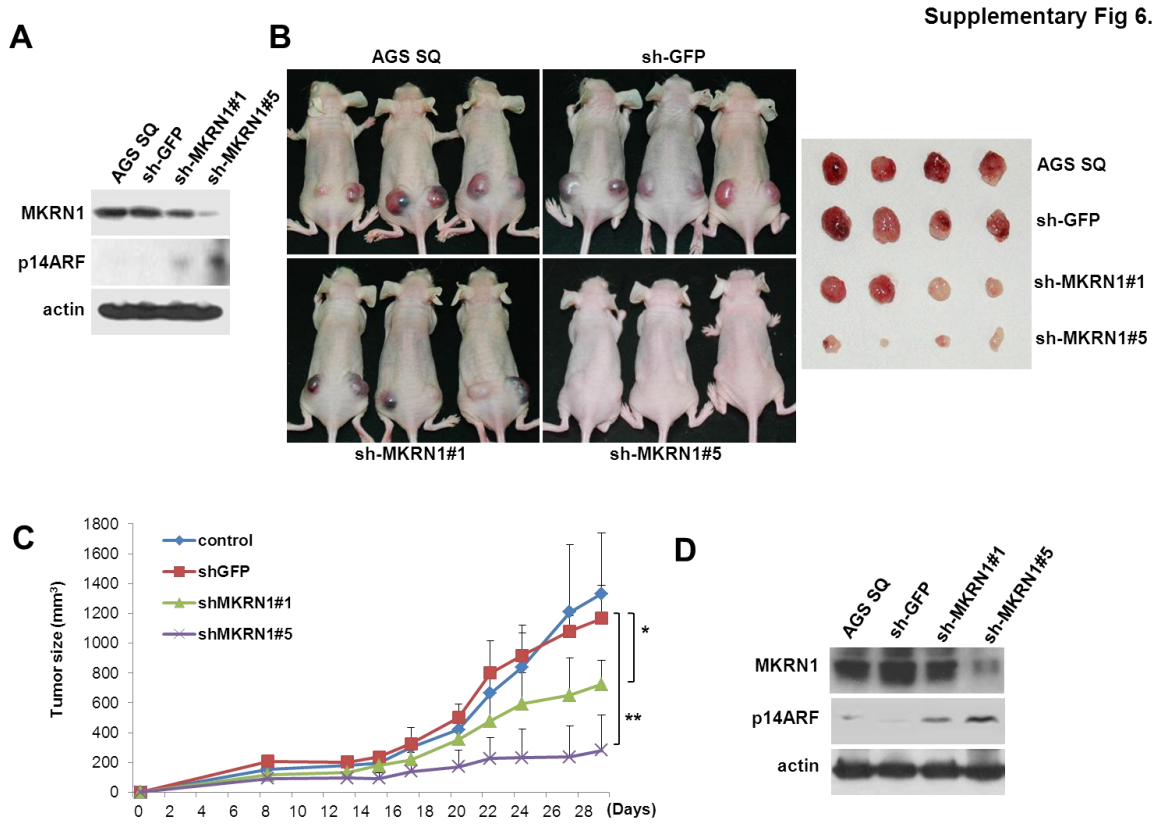


Supplementary Figure 4. Mouse MKRN1 (mMKRN1) induces p19ARF degradation. (A, B) Mouse p19ARF and mMKRN1 interact with each other. Mixtures of plasmids expressing HA/mMKRN1, p19ARF/FLAG, or mock were transfected into 293 cells. The lysates were immunoprecipitated using anti-FLAG or HA antibodies and precipitated proteins were detected using the same antibodies. (C) mMKRN1 degrades p19ARF in a dose-dependent manner. H1299 cells were transfected with mixtures of plasmids expressing p19ARF/FLAG, HA/mMKRN1 in increasing concentrations, or GFP. The cell lysates were immunoblotted with anti-FLAG, HA, and GFP antibodies. (D) mMKRN1 ubiquitinates p19ARF. H1299 cells were transfected with mixtures of plasmids expressing HA/Ub, p19ARF/FLAG, HA/mMKRN1, or mock as indicated, with or without MG132. The cell lysates were immunoprecipitated with anti-FLAG antibodies and the immunoprecipitates were detected using anti-HA, anti-FLAG, and MKRN1 antibodies. Ubiquitination analyses were performed under conditions of denaturation. MKRN1 = Makorin ring finger protein 1; p19ARF = p19

Alternative reading frame; GFP = Green fluorescent protein.

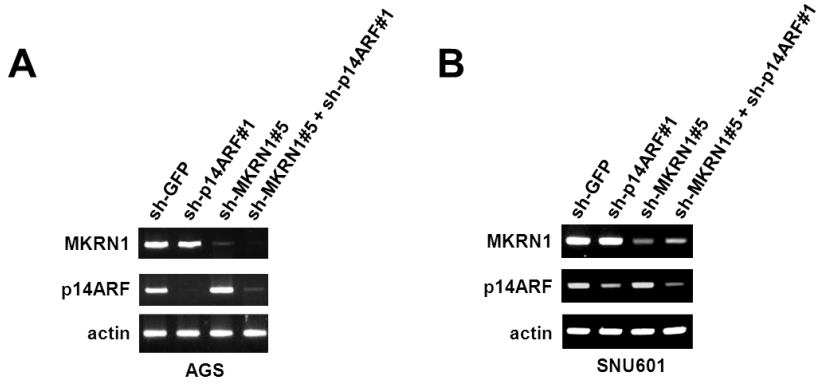


Supplementary Figure 5. Protein levels of MKRN1 and p14ARF are inversely correlated in a variety of gastric cancer cell lines. Extracts of gastric cancer cell lines were analyzed using western blotting and RT-PCR as described in "Materials and Methods". Asterisks indicate cell lines displaying inverse correlations between MKRN1 and p14ARF. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading frame; RT-PCR = Reverse transcription polymerase chain reaction.



Supplementary Figure 6. Depletion of MKRN1 reduced tumor formation in nude mice. (A) MKRN1 knock-down stable AGS cell lines were prepared and protein levels of MKRN1 and p14ARF were detected by western blotting. β-actin was used as a loading control. Stable AGS cell lines were produced using lentiviruses expressing sh-RNAs targeting MKRN1, or GFP. AGS SQ indicates an AGS cell line without lentiviral infection. (B-D) Mice (N=4) were inoculated subcutaneously in both flanks with 10^6 cells of stable MKRN1 knock-down or control AGS cell lines. (B) At 28 days after inoculation, tumor formation in mice and isolated tumors from sacrificed mice were photographed. (C) Quantification of tumor formation by measurement of tumor size 28 days after inoculation (N=4). The error bars indicate 95% confidence intervals of three experiments. All statistical tests were two-sided. (D) Tumors from each group were frozen for lysate preparation and protein levels of MKRN1 and p14ARF were detected by western blotting. β-actin was used as a loading control. *: p =.44, **: p =.22. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading

frame.



Supplementary Figure 7. MKRN1 and p14ARF knock-down in stable AGS or SNU601 cell lines. MKRN1 single knock-down or MKRN1 and p14ARF double knock-down stable AGS or SNU601 cell lines were prepared as described in figure 6 and mRNA levels of MKRN1 and p14ARF were measured by RT-PCR. β-actin was used as a loading control. Lentivirus expressing sh-RNA targeting GFP was used as a control. MKRN1 = Makorin ring finger protein 1; p14ARF = p14 Alternative reading frame; RT-PCR = Reverse transcription polymerase chain reaction, GFP = Green fluorescent protein.